Journal of Environmental Biology *J.Environ.Biol.*
ISSN : 0254-8704 **23(3)**, 301–320 (2002)

Clinical confirmation of trichothecene mycotoxicosis in patient urine.

William A. Croft, Bonnie M. Jastromski, Amanda L. Croft and Henry A. Peters
Environmental Diagnostic Group Inc., Department of Environmental Pathology and Toxicology, 521 Hilltop Drive, Madison Wisconsin 53711 (USA).

(Received : 19 April 2001 ; Accepted : 26 March 2002)

Abstract *: The investigations of four Cases involving mold-contaminated buildings and human reaction to exposure, documents tests of extracted urine containing trichothecene mycotoxins confirming exposure and the diagnosis of mycotoxicosis in humans. In each of four Cases, the urine demonstrated antibiotic activity, sulfuric acid charring, and protein release. Urine was extracted using ethyl acetate 40V/60V[EA]. Extracted mycotoxin spotted on (TLC) displayed color and a range of (rf) between 0.2-0.6 using various solvents. Extract was re-suspended using 50% ethanol V/V to inject mycotoxins into weanling female Sprague-Dawley rats. Degeneration and necrosis of the rat's tissue followed. Koch's Postulates conditions were fulfilled by isolation of the causative agent, the trichothecene mycotoxins and the reproduction of disease. Examination of human tissue within the urine extraction group confirms Koch's Postulates and comparative pathology confirms inhalation Mycotoxicosis, with severe necrosis of the central nervous system and severe scarring within the lungs. Extraction of mycotoxins from human patient urine is a very useful confirmatory test to demonstrate exposure and identify mycotoxicosis. Low concentrations (6%) of sodium hypochlorite were ineffective against the activity of trichothecene mycotoxin. The severity or stages of disease directly correlates the level of exposure or poisoning (Patent Pending).*

Key words *: Trichothecene, Mycotoxicosis, Urine confirmation test, Human pathology*

Introduction

Trichothecene mycotoxins have been implicated in numerous Cases concerning farm animals and human health. The major Case of trichothecene mycotoxins involving humans was noted in an outbreak of Alimentary Toxic Aleukia (ATA) near Orenburg, U.S.S.R. in the early-1940's involving ten percent of the population (Forgacs *et al.*, 1962; Joffe, 1971). Many other reported Cases have involved trichothecene mycotoxins in cereal grains contaminated by trichothecene-producing fungi (Ueno, 1977, 1980, 1983).

Understanding of this poisoning has steadily increased, highlighted by the scientific and medical community acknowledging in 1976 that this poisoning is a "commonly recognized disease" (Ueno, 1977). Identification of inhaled trichothecene mycotoxins in mold-contaminated buildings that poisoned many human inhabitants (Croft *et al.*, 1986) demonstrated another means of exposure.

The medical community including the Surgeon General (Wannemacher *et al.*, 1997) has relied on comparative pathologists to understand the fingerprint of human diseases (Cheville, 1976) and has recognized this disease through chemical and biological weapons, and allergy and asthma epidemics (Johanning, 1999). Mycologists are called upon, to identify mold species in contaminated buildings where human health maladies occur and to determine the mold spore counts (Johanning, 1999). These methods lead to confusion among health officials regarding diagnoses, exposure and safety issues (Burge *et al.*, 2000) especially when under certain conditions the mycotoxin is released as a vapor (Pasanen *et al.*, 1996). Studies have found trichothecene mycotoxins in the urine of animals exposed to toxic molds (Sato and Ueno, 1977). These data indicate that most of the mycotoxin

administered in levels substantially less than LD_{50} is eliminated relatively quickly through the feces and urine (Talmage, 1983; Wannemacher *et al.*, 1997). Higher levels of mycotoxin was associated by interference with elimination via the gastrointestinal tract (0.8%) and in the urine (17%) (Robison *et al.*, 1979).

Trichothecenes are cytotoxic to animals, humans, bacteria, and fungi and have a high affinity for eukaryotic cells (Ueno, 1977). They are known to inhibit protein synthesis at 30 ppb and can affect every cell in the body (Ueno, 1977). Trichothecenes are used as biological warfare agents because they can permanently disable people exposed to them (Talmage, 1983; Wannemacher *et al.*, 1997). The trichothecenes are carcinogenic for man (Costantini, 1998, 1999).

Test conducted on human urine, such as antibiotic tests, sulfuric acid tests, and extractions of mycotoxin placed on thin layer chromatography (TLC) indicate substantial corollaries to the same tests already observed using animal urine. This suggests that urinalysis confirms diagnoses in humans.

This study reports on four “mold contaminated buildings” in which urine was collected from symptomatic people occupying these structures. The signs, symptoms and severity of each were numerically recorded. This allowed for the establishment of the primary signs, symptoms, and precise organs affected. Using this information, the patients were then classified into three stages of the disease. Their urine was assayed, and confirmed to contain trichothecene mycotoxins using TLC reproducing the disease in animals; thus meeting the criteria for Koch’s Postulates. This urine test confirms exposure and the diagnosis of trichothecene Mycotoxicosis. Pathology of a deceased resident defines inhalation compared to ingestion trichothecene Mycotoxicosis and confirms the urine test method for this disease.

Materials and Methods

Case No 1

A family of four, husband 43, wife 40, daughters 10 and 13 years, were living in one of 146 apartments in a complex in Las Vegas, Nevada. The parents moved into the newly constructed apartment and could smell "mold or wet wood", but did not think much of it. Three years later their first daughter was born and eleven months after the daughter’s birth, both daughter and mother were stricken with asthma and hospitalized. Family members suffered chronic malaise, fatigue, severe headaches, asthma, diarrhea, and episodes of various cold and flu strains. The severity continuously escalated, and five and half years later the eldest daughter was rushed to a hospital with severe respiratory distress, hypoxemia, pneumothorax, pneumomediostinum and severe reactive airway disease. The daughter underwent surgery for necrotizing sinusitis (affecting the orbital bone). The daughter’s physician recommended she be taken to a hospital in Denver, Colorado to determine the cause of her illness; the daughter required constant neubulizer treatments.

All tenants within the 146-unit apartment complex suffered from burning eyes, severe headaches, respiratory difficulty, depression, diarrhea, severe chest tightness, nasal burning, leg cramps, dental problems and skin rashes. The husband had three surgeries for the removal of 15 to 20 growths of skin cancer (fibrosarcoma and basal cell carcinomas). Two tumors were 10-15 cm in diameter, requiring skin grafts. The father’s cancer surgeon remarked that he was too young to be developing skin cancer by any normal circumstance.

The family’s apartment flooded every few months due to inadequate plumbing maintenance and blocked drainpipes. This allowed mold to colonize and proliferate. The parents used a Hepa air-filtering unit to clean the air in their apartment. Within three weeks, spores clogged the filter. Several months later, the family moved out of the contaminated apartment.

The family's clothes and belongings were washed and flushed with 6% sodium hypochlorite (bleach) as recommended by the EPA. The family moved to a clean non-contaminated apartment away from the apartment complex. The family members health improved and three months after moving out, a Patch Test (alcohol dehydrogenase activity) (Higuchi *et al.,* 1987) was administered. It indicated a significant decrease in Alcohol enzyme activity, suggesting that the mycotoxin was still within their environment. Urine samples from the four family members were collected.

Case No. 2

This Case involves a family of four in Clearview, Kentucky, husband 43, wife 40, sons 7 and 8 years old. The family's home caught fire, resulting in substantial fire and water damage. Burned debris and water collected in sub-floor ventilation ducts, and was never adequately cleaned. Several months after the fire, the house was repaired, with the exception of the sub-floor ventilation ducts. The family moved back into the house several months later. When the weather cooled and the heating system was utilized an extremely foul-smelling odor made the house uninhabitable. The sub-floor ventilation ducts were contaminated with mold-laden debris, the source of the mycotoxin. The ducts were subsequently replaced, but the home was not cleaned. The family moved back into their home, unaware of the fungal spores contamination.

The family's health deteriorated over the next six years. The mother was diagnosed with rapid heartbeat and severe asthma and she was given a few months to live. The father suffered numerous psychological manifestations including agoraphobia. The young boys could not tie their shoes or ride their bicycles very well and endured an overall stunted development.

All family members suffered the reported signs and symptoms typical of mold exposure (Croft *et al.,* 1986). Their poisoning involved the respiratory system with severe congestion of lungs and sinuses as well as cerebellar disease (Best, 1961). All the members expressed flu-like symptoms, nausea, vomiting, bloating, abdominal distention and pain, excessive flatus, and moderate diarrhea. The children reported having diarrhea accidents in school. All reported skin rashes and infections. The father reported severe hyperemia on the palmer surface of his hands. Moderate to severe malaise and fatigue caused them to sleep 16-18 hours per day while unable to control their body temperature as they reported their home temperature at 85^{o}F. They all reported easy bruising, allergies to molds, and extended bleeding of skin cuts. They had skin edema but no hemorrhaging within the skin. Each parent reported 10 to12 epithelial skin growths on the body ranging from 0.3-0.6 cm in diameter.

Patch tests were conducted and urine samples were taken from each family member. Bulk mold samples were taken from ceiling fan blades loaded with sticky dust build up. Dust samples were submitted for mold spore identification. Active mold growth sites within the house were only detected on damp windows and under the refrigerator. The home had a moldy odor. The author experienced a burning sensation of the skin upon exposure.

Case No 3

A 55-year-old female moved into a newly constructed apartment in DePere, Wisconsin. She was essentially free of disease with no allergies, but experienced a gradual decline in her health over the next 12.5 years. A few months after moving into her apartment, she developed severe immune suppression, resulting in a mononucleosis infection, which produced severe liver damage, preventing her form working.

The woman's health continued to decline, as did her mental processes. She suffered from partial loss of memory and balance, severe asthmatic congestion of her lungs, and severe bruising, degeneration and hyperemia of the skin. She had multiple areas of scarring from intense itching on her arms. She developed two skin growths 2.1cm in diameter near both breasts. She expressed other signs and symptoms typical of

mold spore exposure as reported (Croft *et al.*, 1986). She also developed severe chemical hypersensitivity. When shopping in malls or when in an apartment, she would experience adverse reactions resulting in falls and injuries including fractures. She and her physicians were unaware of the reason for the decline in her health. She was not aware of the extent of her illness until she vacated the contaminated apartment.

Her apartment was well maintained with no obvious water damage. Close examination of the heating and cooling system revealed that a cold return duct had been installed with no moisture barrier, causing water to condense. The insulation supported heavy mold growth, which also grew into a supporting wall. Bulk samples were taken for microbiological identification. The Patch test was administered and a urine sample was collected.

Case No. 4

The fourth Case concerned 502 tenants living in an apartment located in Manhattan, New York. The apartment complex consisted of a tower of 33 floors and a lower unit of 14 floors. Examination of this apartment complex revealed it to be stuffy and irritating to the skin. Additionally, the apartments had a nauseating mold odor, high humidity and active aggressive mold growth. Seventy-three apartments in which the residents were living were examined. The mold growth was so substantial that bulk mold samples were taken for identification with ease using forceps. Mold growth was observed in most rooms of each apartment, the heaviest growth being in bathrooms and kitchens. Apartment doorsills, window shades, hallways, elevator shafts and ventilation duct covers were heavily contaminated with black mold spores. Black mold spores were detected drifting across the floor by elevator doors while the elevator was descending. Outside walls were also open to water damage and mold growth as rain and wind penetrated the walls. Water was reported running onto apartment floors during rainstorms. During the investigation of the apartment complex, the author experienced burning of the face and eyes. The 502 tenants living in the apartment complex reported many degenerative diseases, various cancers, unexpected deaths and signs and symptoms typical of mold exposure as reported (Croft *et al.*, 1986). One or more residents of each of the 72 apartments examined were photographed and administered a Patch test. Signs, symptoms and a brief medical history were recorded. This method was important in establishing the causative agent of the residents' illnesses. Areas within each apartment showing mold growth were photographed. Twenty-one residents, each from a different apartment, were randomly selected and a urine sample was collected. Three of the 21 residents who had provided a urine sample subsequently died. An autopsy could be performed on only one of residents. The tissue recovered from the autopsy was examined for pathological changes.

The fingerprint of the illness was determined by acquiring the specific signs, symptoms and a brief medical history for each Case. Comparative pathology and direct pathology, which are the bases for studying human disease (Cheville, 1976), were also used to gain additional understanding of inhalation Mycotoxicosis. This is the usual method utilized in finding the fingerprint and establishing the causative agent for an illness within humans (Peters *et al.*, 1984; Croft *et al.*, 1986). The degree of illness or poisoning was determined by administration of a Patch test. The family members and any ambient environmental conditions associated with the illness were photographed.

William J. Rea, M.D. and staff at the Environmental Health Center (EHC) in Dallas, Texas (EHC-D) completed a medical work up of each patient for Cases 1 and 3. Theodore Simon, M.D. completed a single photon emission computed tomography (SPECT) of the brain using technetium – 99. Nancy A. Didriksen, Ph.D. evaluated neurocognitive and personality / behavioral concomitants of toxic exposure, completed neuropsychological testing. The tests

completed were : The Halstead Category test, a Comprehensive Neuropsychological Screen, The Benton Visual Retention Test, The Symbol Digit Modalities Test, The Beck Depression Inventory-II, a Profile of Mood States, a Wide Range Achievement Test, The Health and Wellness Attitude Inventory and Symptom Checklists for families. Henry Peters, M.D. Department of Neurology, at the University of Wisconsin Hospital and Clinics, administered a neurological examination to a patient in Case No.3. Dr. Peters, a highly qualified environmental neurologist has examined several families exposed to trichothecene mycotoxins, including the family reported in (Croft *et al.,* 1986). Scott Prince M.D. MSPH of the University of Kentucky, Chandler Medical Center performed a medical work-up on the family members in Case No. 2. Numerous Medical specialists within the New York City hospital complex and Irene Grant, M.D., infectious disease specialist, also located in Manhattan, performed medical workups for Case No. 4.

The Patch Test was conducted as described (Higuchi *et al.,* 1987) and used to determine the amount of alcohol dehydrogenase (ADH) activity, trichothecene mycotoxins (epoxides) inhibit (ADH) enzymes (Ueno, 1977). A round cotton ball (1 cm) was dipped into 70% non-denatured ethanol and placed on a medial surface of the upper arm and held by the patient for 7 minutes. The patch was then removed and the area was observed for 10 minutes. The degree of (ADH) inhibition was determined on a scale of 0 to 5, with 5 having the greatest severity of inhibition. A **score of zero** indicated no reaction in the area, 7-10 minutes after removal of the cotton ball. Where a burning sensation, redness or edema of test area was observed after cotton ball was removed, scores were recorded as follows : **(Score1)** seven minutes after removal of cotton ball (**Score2)** five minutes after removal of cotton ball (**Score3)** three minutes after removal of cotton ball (**Score4)** one minute after removal of cotton ball (**Score5)** burning sensation, redness, or edema of test area before removal of cotton ball. In severe Cases only, edema is detected due to degeneration of skin. The estimated accuracy is (±) 1.

Urine samples were observed for volume, antibiotic activity (bacterial growth, or no growth) (Pathre and Mirocha, 1977; Ueno, 1977), protein content and reaction with sulfuric acid. The sulfuric acid reaction with the urine was determined by spotting several drops of each sample on a TLC plate and allowing it to dry. One drop of concentrated sulfuric acid was then applied to each spot. The intensity and color of the reaction spot was observed and scaled from 1-3 as follows : (1) light and gray in color (2) moderate and brown in color (3) intense and black in color. If the sample contained no mycotoxin, no color change was observed. The estimated accuracy is (±1) (Pathre and Mirocha, 1977).

The urine samples collected were extracted for trichothecene mycotoxins using the volume ratio of ethyl acetate 60V/40V to urine. The volume of urine collected and extracted ranged from 215-3,700 ml. The ethyl acetate fraction was taken to dryness and the extracted mycotoxin (dark brown-black gummy material) was transferred to a 10 ml vial using 50% non-denatured ethanol and dried using a warming plate. It was then resuspended using 50% non-denatured ethanol. A ratio of 1 ml of ethanol to 300 ml of initial urine extract was found to be the proper level of exposure to mycotoxin. This ratio allowed the test animals to live for ten days, giving time for the pathology to develop within their organs. This method was determined by several preliminary injections causing acute toxicity. Patients from Case No. 4 had approximately three times the amount of observed extracted material in the vial than the other Cases. The dilution ratio was reduced to a level of 1 ml of ethanol to 100 ml of initial urine extract based upon animal injection exposure.

This dilute mycotoxin extract was injected twice intraperitoneally into weanling female Sprague-Dawley rats (weight range 29-31g). Injections of 0.25 ml were completed on the first and fourth day of the study. Animals were provided standard rat pellets and tap water in

bottles ad libitum and observed daily. The animals were weighed on day 1 and again at necropsy. The animals were allowed to live 10 days and were then euthanised, by exposure to carbon dioxide, and necropsied. Animals that died before that time were necropsied. The animal tissues were placed into 10% buffered formalin, processed into histological slides, and stained with Hematoxylin and Eosin. The tissues were examined using a light microscope. The author's urine was used as the negative control sample and extracted as the other urine samples completed in this study. The control rats were kept from the other treated rats and injected with the negative control urine in a 1ml non-denatured ethanol to 300 ml urine ratio.

H.B Schiefer, DVM, PhD, and J. Smits, DVM, PhD, Medical Pathologists, of The University of Saskatchewan, Saskatoon, S7N 5B3, Canada confirmed that the HEPA filter, from 1 family's apartment (Case No. 1) as trichothecene mycotoxin, using laboratory rats, fulfilling Koch's Postulants. Therefore, the (Case No. 1) family served as the positive control for the other patients in the study.

The same diluted mycotoxin extract injected into the rats was also spotted on a thin layer chromatography (TLC) plate TLC-plastic sheet silica gel, 60 pre-coated 20x20 cm, 0.2cm layer thicknesses were obtained from DC-Plastikfolien Kieselgel 60, Germany. The extract color and relative frequency (rf) observed was consistent with those reported in the literature for trichothecene mycotoxins (Pathre and Mirocha, 1977).

Jack Charney MD, of Beth Israel Medical Center, Department of Diagnostic Pathology and Laboratory Medicine, New York City, performed the gross dissection (autopsy). Susan Morgello, MD, of Mount Sinai School of Medicine, Neuropathology, of The Mount Sinai Hospital, New York City, examined the brain and spinal cord of a deceased resident from Case No. 4. The resident was a 63-year-old African American male who was one of the 21 residents in which urine was sampled, and served as the positive control. The tissues were placed into 10% buffered formalin, processed into histological slides, and stained with Hematoxylin, Eosin and Trichome (lung sections). The tissues were examined using light microscopy.

Initial mold samples were examined by direct spore identification using a light microscope to determine the species of mold present. The growth of sample spores was then allowed for specie determination of each.

Many signs and symptoms are associated with disease resulting from mold exposure. Fifty of the most common symptoms were chosen for Cases 1-4 and were scored and listed according to severity (Tables-1 and 2). Scoring of the sign or symptom was performed using the following scoring system : **(0-1)** mild or not present, (**2-3)** moderate and (**4-5)** severe. The estimated accuracy is ± 1. A total of the scores for the signs and symptoms of each patient was obtained and used to Stage the disease for each subject (Tables-1 and 2). Of the four Cases studied, scores ranged from 128 to 217.

The three Stages (1-3) ranging from lower to higher severity of poisoning were modified according to exposure via the air as opposed to ingestion already established (Forgacs *et al.*, 1962; Joffe, 1971). A separate Stage of convalescence occurs when a patient is completely removed from the contaminated premises and the source of mycotoxin or mold spores.

Stage 1 : The primary changes are in the brain, respiratory and immune systems, mucus membranes and gastrointestinal tract. Signs and symptoms may include burning sensation in the mouth, tongue, throat, palate, esophagus, and stomach, which is a result of the action of the toxin on the mucous membranes and skin in the exposed areas. Moist areas of the body armpits, under breasts, belt line and groin are more sensitive or first affected. Patients may report burning within the eyes, ears and nose. Patients also reported that their tongues felt swollen and stiff. Mucosa of the oral cavity may be

hyperemic. Mild gingivitis, stomatitis, glositis, and esophagitis developed. Inflammation, in addition to gastric and (small and large) intestinal mucosal, resulted in vomiting, diarrhea and abdominal pain. Excessive salivation, headache, dizziness, weakness, fatigue and tachycardia were also present.

There may be fever and sweating. The respiratory system develops burning sensations and congestion. Severe exposure to mycotoxin within the lungs may lead to congestion, edema and failure, due to caustic action. Body temperature remains normal and controllable by the patient. The poisoning appears and disappears relatively quickly in this Stage with the exception of, lungs and central nervous system. Initially (Stage 1), the patient's symptoms are very uncomfortable or painful. As the poisoning continues and the patient progress toward Stage 2, he or she becomes accustomed to the presence of the mycotoxin and a quiescent period follows due to lack of nerve sensation. Depending on exposure levels, the first Stage may last from 3 - 9 days. In scoring the 50 signs and symptoms listed in Tables-1 and 2, an average score range of 20-45 represents Stage 1.

Stage 2 : This Stage is often called the latent Stage or incubation period because the patient feels apprehensive, but is capable of normal activity in the beginning of this Stage. Every organ of the body is affected by degeneration and necrosis with continued exposure. The primary target organs for an individual become evident over time, due to biological variation. These are disturbances in the central and autonomic nervous systems resulting in headaches, mental depression, loss of short-term memory, loss of problem-solving ability, various neuropsychiatric manifestations, meningism, severe malaise and fatigue, narcolepsy, loss of temperature control, hyperesthesia or numbness of body areas, and cerebellar dysfunction including hypotonia, attitude and gait, dysmetria, asthenia, vertigo, disturbances of speech, and loss of balance (Best, 1961). Spinal cord degeneration may also be observed in gait and reflex abnormalities, such as the ability to drive vehicles, ride bicycles or pass sobriety tests (inability to tolerate ethyl alcohol). Attention deficient disorder may be observed in children. Various systems may include : **Eyes** : visual disturbances, floating objects, light sensitive, lack of tears, burning and itching. **Ears** : burning, itching, and loss of hearing. **Immune and hematopoietic** : progressive loss of white and red cells including a decrease of platelets and hemoglobin, and high susceptibility to bacterial, mycotic and viral infections, debilitating chemical and allergies. **Gastrointestinal** : metallic taste in mouth, tooth loss, gum problems, stomatitis, sores in gums and throat, nausea, vomiting, diarrhea or constipation, excessive flatulence, abdominal distention, hepatitis, pancreatitis, and diabetes mellitus. **Respiratory :** burning and bleeding from nasal membranes, respiratory difficulty, asthma, extreme susceptibility to cold, flu and pneumonia. **Skin :** thinning of hair on head, burning on face, rashes, irritation, and edema. **Renal** : proteinuria, possible hematuria. **Reproductive :** irregular ovarian cycles, increased menstrual flow, fibroid growths in uterus, cystic development in mammary glands, and tumors of mammary and prostate glands. **Musculoskeletal** : somatitis, muscle weakness, spasms, cramps, joint pain, enlargement of joints in hand, and clubbing of fingers. **Cardiovascular :** chest pain, palpitations, ruptures of atrial walls, myocardial infection and aneurysm of arteries.

The skin and mucous membranes may be icteric, pupils dilated, the pulse soft and labile, and blood pressure may decrease or increase. The body temperature does not exceed 38 degree C and the patient may be afebrile, or chilled. Visible hemorrhagic spots may appear on the skin. Thoughts of suicide may be prominent in the person's mind at this time or anytime in Stage 2. Human bonding is very important for survival.

Degeneration and hemorrhages of the vessels marks the transition from the second to the third Stage of the disease and may not be consistently observed. The degeneration of the vital organs including serious respiratory insufficiency or asthma and CNS degeneration

will take the patient into Stage three along with development of necrotic angina. If exposure continues, depending on exposure levels, Stage 2 may continue from weeks to months or even years until the symptoms of the third Stage develop. Evaluating the 50 signs and symptoms (Table-1 and 2) by assigning a score (0-least intense to 5-most intense or severe) to each symptom, we have determined that an average score range of 45-180 represents Stage 2.

Stage 3 : Severe degeneration of the vital organs. The transition from the second to the third Stage is sudden. In this Stage, the patient's resistance is already low, and violent severe symptoms are present, especially under the influence of stress, or associated with physical exertion and fatigue. The first visible sign of this Stage may be lung, brain or heart failure (heart attack), with or without the appearance of petechial hemorrhage on the skin of the trunk, the axillary and inguinal areas, the lateral surfaces of the arms and thighs, the face and head, and in serious Cases, the chest. The petechial hemorrhages vary from a few millimeters to a few centimeters in diameter. There is increased capillary fragility and any slight trauma may cause the hemorrhages to increase in size.

Aneurysms of the brain or aorta may be observed by angiography. Hemorrhages may also be found on the mucous membranes of the mouth and tongue, and on the soft palate and tonsils. There may be severe interstitial thickening or scarring of the lungs, or respiratory failure. Nasal, gastric and intestinal hemorrhages and hemorrhagic diathesis may occur. Necrotic angina begins in the form of catarrhal symptoms and necrotic changes soon appear in the mouth, throat, and esophagus with difficulty and pain on swallowing. Severe degeneration of the skin on the face, eyelids, and loss of lashes is also often present.

Necrotic lesions may extend to the uvula, gums, buccal mucosa, larynx, vocal cords, lungs, stomach, and intestines and other internal organs such as the liver and kidneys and are usually contaminated with a variety of avirulent bacteria. Bacteria infection causes an unpleasant odor from the mouth due to the enzymatic activity of bacteria on proteins. Areas of necrosis may also appear on the lips and on the skin of the fingers, nose, jaws, and eyes. Regional lymph nodes are frequently enlarged. Esophageal lesions may occur and involvement of the epiglottis may cause laryngeal edema and aphonia (loss of voice). Death may occur by strangulation.

Patients may suffer an acute parenchymatous hepatitis accompanied by jaundice. Bronchopneumonia, pulmonary hemorrhages, and lung abscesses are frequent complications. Tumors may develop of various organs, including skin, urinary bladder, brain, mammary gland, bone, immune, liver, prostate, possibly resulting in death. The most common cause of death is brain failure due to both direct effects of the mycotoxin on the central nervous system and indirect effects due to respiratory failure or lack of oxygen to the brain caused by the severe caustic inflammation (fibrinous exudation) reaction with the lung tissue, rendering it non-functional. Again, using the scoring system represented in Tables-1 and 2, an average score of greater or equal 180 represents Stage 3.

Stage of convalescence : The course and duration of this Stage 3 depends on the intensity of the poisoning and complete removal of the patient from the premises or source of mycotoxin. Therefore, the duration of the recovery period is variable. There is considerable cellular necrosis and scarring to all major organs of the body in which cells will not regenerate, including the brain, spinal cord, eyes, lung, heart, liver, pancreas, kidney, adrenal, and blood vessels. If the disease is diagnosed during the first Stage, hospitalization is usually unnecessary, but allergies and asthma should be monitored closely. If the disease is diagnosed during the second Stage and even at the transition from the second to third Stages, early hospitalization may preserve the patient's life. If however, the disease is only detected during the third Stage, death cannot be prevented in most Cases.

Immune therapy for allergies to molds and chemicals is another important part of treatment of this disease during this time. Mold patients that have been completely removed from the mycotoxin contamination will usually respond to mold exposure in 2-4 weeks, expressed as skin edema or wheals. Extreme caution is recommended due to possible anaphylaxis. Patients in Stage 2 require three months of supportive therapy before alcohol dehydrogenase (ADH) activity returns to normal levels and the mycotoxin influence leaves the body. Establishing the will or purpose to live and emotional support is very important therapy for patients in this Stage.

A statistical analysis of the 50 signs and symptoms, which were identified into 6 classes and attributed grades of 0-5 was performed. Zero represents the deceased individuals. The data in the 4 Cases were compared using X2 (Chi Square) (Steel and Torrie, 1960).

Results

Case No. 1

The health of the family members living in a Las Vegas, Nevada apartment complex was severely altered by exposure to mold in their contaminated apartment as documented by William J. Rea, MD, Environmental Health Center of Dallas, Texas. Skin testing clearly demonstrated mold exposure, as severe respiratory difficulty was reported. The parents and youngest daughter were Staged at a high 2. The oldest teenage daughter nearly died because of her severe asthma and was placed at Stage 3. Dr. Simons' SPECT Scans of the brain of all the family members revealed temporal asymmetry with mismatched activity, patterns which have been seen in patients with neurotoxicity. Neuropsychological evaluation by Dr. Didriksen revealed moderate impairment in executive functions including, problem-solving, judgment, abstract reasoning, concept formation, mental efficiency, and new learning, the skills most needed for effective and efficient functioning in everyday life. Visual-motor-spatial integration skills were below average and cerebral changes were observed. Depression associated with irritability, fatigue, hypersomnia, difficulty concentrating and agitation were also reported. The oldest daughter had attempted suicide with no success. The mother had reported that she was living only to care for her children. Several parents reported that their children developed Attention Deficit Disorder (ADD) who lived within the contaminated apartment complex.

Family members were very reactive to the Patch Test with a score of 4. Penicillium (45%), Cladosporium (20%), Trichoderma (20%), and Stachybotrys Chartarum (atra) (15%) were the principal mold spores identified from the HEPA filter removed from their apartment (Stetzenbach, L.D., University of Nevada-Las Vegas). The urine volumes collected were as follows : father-2 150 ml, mother-850 ml, oldest daughter-340 ml, youngest daughter-394 ml. Urine samples exhibited no bacterial growth and no putrefaction at room temperature for five months,

Chart 1 : Summary of signs and symptoms displayed in Cases 1-4.

Cases:	X^2
All died	0.0
Case 1 LV	10.8
Case 2 KY	10.8
Case 3 WI	6.58
Case 4 NY 2000	9.33
Case 4 NY 2001	4.2*

*Three New York residents that were living in this building have died.

demonstrating antibiotic activity. The color of the urine went from a yellow to a tan color. Excessive amounts of protein were observed in the bottom of the urine container.

The sulfuric acid test of the urine revealed dark brown, black coloring (scale of 2-3) indicating mycotoxin present. Urine collected and extracted for mycotoxin revealed substantial levels of mycotoxin, which was injected into the weanling rats. On TLC, the extract color and relative frequency (rf) (0.2-0.6 various solvents) were consistent with those reported in the literature for trichothecene mycotoxins (Pathre, Mirocha, 1977). 10.2 grams of black, syrupy material was submitted to (Drs. Schiefer and Smits, University of Saskatchewan, Saskatoon S7N 5B3, Canada), who confirmed, via animal testing, that the material contained high levels of trichothecene mycotoxins.

Mycotoxin extracted from the HEPA filter was spotted on thin layer chromatography and treated with 6% sodium hypochlorite (bleach) solution. The sodium hypochlorite solution had no effect on the spotted mycotoxin (0.5-1.5 μg/spot) compared to untreated mycotoxin and the relative frequency (rf, 0.43) did not change. When full strength sodium hypochlorite was applied to the spotted mycotoxin on the chromatograph at the site of origin, the mycotoxin was destroyed. The average score for the intensity of the signs and symptoms for the Las Vegas family is 153, placing them in Stage 2.

Case No. 2

The results for the family in Kentucky clearly demonstrated health changes, obstructive respiratory infections, recurrent papular rash, and decreased energy levels. The mother also developed ventricular tachycardia, reported by Dr. Scott Prince, M.D. The family members were exposed to Penicillium sp. and Cladosporium sp. that were detected in the cold air return duct within their home (Dr. T.J Passon, Pure Earth Environmental Lab. Inc. Pennsauken, New Jersey).

The family members were severely affected. The mother was at Stage 3 because of severe asthma and heart changes. Doctors reported to her that her life expectancy might be severely shortened as a result of exposure. The father and two young boys were Staged at a high 2. They suffered severe mental changes including cerebellar changes. The family members could not control body temperature. All members of the family responded strongly to the patch test with a scale of 4. Urine volumes from family were as follows; father-1,380 ml, mother-600 ml, oldest son-700 ml, and youngest son-740 ml. The urine collected from each member was observed to have no bacterial growth, suggesting antibiotic properties. The sulfuric acid test revealed a dark brown, black, color (scale 2-3), which indicated trichothecene mycotoxin. Extracted mycotoxin spotted on (TLC) displayed spots and a range of (rf) of 0.2-0.6 using various solvents, which was consistent with those reported in the literature for trichothecene mycotoxins. The mycotoxin extracted from each urine specimen was injected into weanling rats. The observed pathology matched the signs and symptoms reported. The average score for the intensity of the signs and symptoms for the Kentucky family is 153, Stage 2.

Case No. 3

The Case involving a female living in an apartment in DePere, Wisconsin, clearly demonstrated severe poisoning from this disease over the course of, 12.5 years. She had severe edema, severe hyperemia, degeneration of the skin, petechial bleeding and loss of sensation on her body. She had severe bruising of the feet, arms and body, related to contact with objects. She expressed severe congestion of her lungs, severe necrotic sores in her mouth and subcutaneous hemorrhage over her body, placing her into Stage 3 of this disease as confirmed by Dr. Rea EHC-Dallas, Texas. Dr. Simon observed Temporal Asymmetry, patterns which are observed in patients with neurotoxicity. Dr. Didriksen reported neurocognitive test results indicating moderate impairment of brain-related

abilities, overall, with impairment on individual tests ranging from mild to severe. Loss of short-term memory was severe and was the ability to recall the shapes of blocks of the Tactual Performance Tests (incidental memory). Specific neurocognitive deficits, both sensory and motor were observed. Greater dysfunction was observed in the right hemisphere. Depression and concern with ill health were very significant and were consistent with others exposed to molds and examined by Dr. Didriksen's.

Dr. Peter's examination of the patient revealed she had difficulty with rashes on the skin, feet and lower legs. Skin problems improved when she was away from the apartment although she continued to complain of impaired in balance and weakness. On examination the cranial nerves were intact including ocular motor movements, and visual fields to confrontation. On Romberg she was unsteady during the procedure. Strength of upper extremities was good with no grasp reflex and slowed rapid alternating movements. There was no drift. There was cog wheeling bilaterally at the elbows. Strength was equal in the lower extremities and unsteadiness on Romberg. Deep tendon reflexes were equal and there was no Babinski reflex sign. The patient did have some difficulty with estimation of position sense in the fingers.

Dr. Morris reported this patient is severely allergic to molds, yeast, phenol, benzoic acid, ethanol and formaldehyde. Emotional support was very important to this women's surviving this severe mold exposure as reported by Dr. Morris. *Penicillium* sp. and *Cladosporium* sp. molds were isolated and identified within her apartment (Passon, T. J., Pure Earth Environmental Lab. Inc. Pennsauken, New Jersey).

She could not feel the Patch test, but reacted with raised edema of the skin in the test area (score of 5). The urine was collected over 36 hours to a volume of 3,700 ml, and demonstrated no bacterial growth over a period of 5 months, showing antibiotic activity. The urine reacted to sulfuric acid by turning dark brown, black (scale of 2-3), indicating trichothecene mycotoxins were present. Extracted mycotoxin spotted on (TLC) displayed a color and a range (rf) of 0.2 - 0.6 using various solvents, which were consistent with those reported in the literature for trichothecene mycotoxins. Severe amounts of protein were observed in the urine sample. Extracted mycotoxin from the urine was injected into weanling rats and clearly demonstrated degeneration and necrosis of the rats' organs that matched her signs and symptoms, e.g., this patient used artificial teardrops and the rat's tear glands were found to be degenerative and necrotic. In Table-1, the score for the intensity of the signs and symptoms for the women in Wisconsin is 198, which places her in Stage 3. This woman has presently vacated the contaminated premises and is undergoing immune therapy with Dr. Morris for her allergies.

Case No. 4

This Case, involving a large apartment building in Manhattan, New York, revealed that *Cladosporium*, *Penicillium*, *Stachybotrys* sp. and other mold species were present. The Patch test was conducted on all 73 individual families examined, with all falling within a range of 3-4 (indicating a positive reading). The residents remain living in a heavily contaminated environment and are expressing signs and symptoms of severity that Stage their poisoning at a 2. With continual exposure to the mycotoxin, the residents can move into Stage 3 at anytime. Some of the residents have already been staged at level 3.

Dr. Irene Grant verified signs and symptoms of residents. The average score for the 21 residents is 171 for the year 2000, which places them in Stage 2. The residents were interviewed approximately one year later, their signs and symptoms were re-scored. Their average increased to 191.

The urine volumes collected were as follows : (1-1,610 ml, 2-1,240 ml, 3-1,200 ml, 4-930 ml, 5-1,660 ml, 6-1,070, 7-700 ml, 8-1,730 ml, 9-610 ml, 10-400 ml, 11-800 ml, 12-330 ml,

13-800 ml, 14-800 ml, 15-815 ml, 16-650 ml, 17-800 ml, 18-810 ml, 19-825 ml, 20-700 ml, 21-215 ml. The author's urine was collected as a negative control as 22-945 ml. The 21 different residents' urine showed no bacterial growth, indicating antibiotic activity, and marked amount of protein was observed. The sulfuric acid tests ranged from gray to black (showing charring of the mycotoxin) and scoring 1-3 on the scale reflective of urine volume and concentration. Extracted mycotoxin spotted on (TLC) displayed a color and a range of (rf) of 0.2-0.6 using various solvents, which was consistent with those reported in the literature for trichothecene mycotoxins. Out of the 21 patients whose urine was sampled, three have died,

Table 1 : Summary of signs and symptoms arranged in order of intensity according to the average score from each case. The average for 2001 and the deceased are included for case 4.

S. No.	Number of people averaged	4	4	1	21	19	3
	Signs and Symptoms	**Average Case 1 Las Vegas**	**Average Case 2 Kentucky**	**Average Case 3 Wisconsin**	**Average Case 4 (2000) New York**	**Average Case 4 (2001) New York**	**Average Case 4 Deceased**
1	Mental Depression	5	5	5	5	5	5
2	Narcolepsy	5	5	5	5	5	5
3	Severe malaise and fatigue	5	5	5	5	5	4
4	Severe headaches	4	4	5	5	5	5
5	Heart, chest pains	4	4	5	5	5	4
6	Loss of temp control, chills	3	5	5	4	5	5
7	Loss of Balance	4	4	4	5	5	5
8	Attitude changes	4	4	4	5	5	4
9	Loss of short term memory	4	4	4	4	5	5
10	Intolerance to ethyl alcohol	4	4	5	4	4	4
11	Skin irritation and rashes	4	4	5	4	4	4
12	Various Neuropsychiatric Manifestations	3	4	5	4	4	5
13	Diarrhea	4	4	4	4	4	4
14	Dysmetria, hypermetria (legs)	4	4	4	4	4	4
15	Loss of problem solving	4	4	4	4	4	4
16	Hemorrhage(nose, gums, throat)	3	3	5	4	4	5
17	Muscle spasms and cramps	3	3	4	4	5	5
18	Stomatitis, sores in mouth	3	3	5	4	4	5
19	Lungs, Asthma	4	3	5	4	4	3
20	Meningism, pain, surface brain pain	3	4	5	3	4	4
21	Allergies	3	3	5	4	4	4
22	Debilitating chemical hypersensitivity	3	3	5	4	4	4
23	Hyperesthesia or numbness of body areas	4	4	3	4	4	4
24	Metallic taste in mouth	3	3	5	4	4	4
25	Protein in urine	3	3	4	4	5	4
26	Visual disturbance (floating object, light sensitive)	3	3	5	3	4	4
27	Burning face	3	3	4	4	4	4

Continued….

S. No.	Number of people averaged	4	4	1	21	19	3
	Signs and Symptoms	**Average Case 1 Las Vegas**	**Average Case 2 Kentucky**	**Average Case 3 Wisconsin**	**Average Case 4 (2000) New York**	**Average Case 4 (2001) New York**	**Average Case 4 Deceased**
28	Burning nasal membranes	3	3	4	4	4	4
29	Joint pain	3	3	4	4	4	4
30	Nausea	3	3	4	4	4	4
31	Severe flatus, gas	3	3	4	4	4	4
32	Tooth loss, gums problems	3	2	5	4	4	4
33	Vertigo	3	3	4	4	4	4
34	Skin edema	3	3	5	3	4	3
35	Burning and itching eyes	3	3	3	4	4	4
36	Somatitis, sore muscles	3	3	3	4	4	4
37	Severe muscle weakness	2	2	4	4	4	5
38	Loss of hearing	2	2	4	3	4	4
39	Thinning of hair	3	3	3	3	3	3
40	Burning in Ears	2	2	3	3	4	4
41	Surgeries	2	1	5	2	2	5
42	Fever	2	2	2	3	4	3
43	Susceptible to bacterial, viral, mycotic infections	2	2	2	3	4	3
44	Vomiting	2	2	2	3	3	3
45	Menstrual flow longer, heavy	4	4	0	2	3	N/A
46	Other tumors or cancers	1	0	0	1	2	5
47	Skin tumors	1	2	5	0	0	0
48	Hematuria	2	2	1	1	1	1
49	Clubbing of fingers	1	1	3	0	1	0
50	Skin Bleeding	0	0	4	0	1	1
	Totals	152	153	198	176	191	189

63-year-old male, with myeloid cancer, a 55-year old female suspected (brain failure) and an 81-year-old female with breast cancer. The average age of the three patients was 63.3 years and represents 14.3% of the 21 residents sampled. The three patients had an average signs and symptoms score of 189, shown in Table-1. The 21 residents had an increase score of 20 after one year in this active mold growth apartment complex, shown in Table 2. Of the three deceased residents, the 63-year-old male was the only one an autopsy was performed. Examination of his tissues, taken at autopsy using light microscopy, revealed severe generalized degeneration and necrosis of neurons and vessels of the brain which included the cerebral hemispheres and brain stem. There was hemorrhage and thrombi detected within the necrotic vessels, there was severe loss (75%) Purkinje cells in the cerebellum. There was no cellular response detected due to necrosis of neurons, typical of chemical effect (epoxide, mycotoxin) in the entire length of the spinal cord, as well as severe loss of (large cells) in anterior and ventral motor neurons (lateral sclerosis). Before death, this patient was observed having difficulty walking. There appeared to be moderate degeneration of the corticospinal tracts. There was degeneration of the dorsal root ganglion and lipid peroxidation of the spinal nerve. In the distal end of the spinal cord, tumor cells were detected in a small site in the central cannel. Examination of the skeletal muscle revealed degeneration and

necrosis. The pituitary gland demonstrated generalized degeneration of the cells with focal necrosis within the posterior section.

The lungs demonstrated severe loss of functional tissue due to severe fibrinous exudation, an inflammatory response and caustic nature of the inhalation of fungal spores containing mycotoxins (Robins, 1969). This non-cellular inflammatory response has lead to a severe thickening (50-100x) and organizing of the interstitial tissue as essentially non-functional tissue as demonstrated and observed using Trichome stain.

The intestines demonstrated severe loss of mucosal villi and degeneration. There was degeneration and focal necrosis of the pancreas. The liver displayed degeneration and focal necrosis of the hepatocytes and chronic passive congestion. There was severe bile duct degeneration as well as fibrosis with bile pigments detected in the bile duct tissue and congestion of bile within hepatocytes. There were degeneration and necrosis of the tubules and glomeruli of the kidney. There was degeneration of the adrenal gland, thyroid, lymph nodes, spleen, skin and bone marrow. The myocardium displayed degeneration, focal necrosis, focal hemorrhage and scarring. The coronary vessel displayed cystic degeneration and focal necrosis. The urinary bladder displayed papillary fibroadenoma and smooth muscle necrosis. The eyes were not taken or examined.

The mycotoxin was injected into weanling female rats from all four Cases and the rats displayed depression, lack of eating, diarrhea, and decreases in spontaneous movement. Their bodies were cold to the touch. Examination of the tissues revealed degeneration and necrosis in all the organs examined including brain, thymus, eye, eye glands, lungs, heart, intestines, lymph nodes, arteries, liver, pancreas, spleen, kidneys, adrenal, dorsal root ganglion, spinal cord and bone marrow, all signs correlating with the signs and symptoms expressed by the family members. The disease was reproduced in these animals. Hemorrhaging and focal areas or ruptured alveoli with focal areas of interstitial thickening (focal areas of fibrinous exudates) were observed within the lung tissue. The negative control rats did not display any degeneration or necrosis as with those observed in the mycotoxin-exposed groups.

Comparing other toxicity studies in rats exposed to the macrocyclic trichothecenes, the LD_{50} values have been reported to be 0.5 - 0.75 mg/Kg. (Sato and Ueno, 1977). The signs and symptoms of acute intoxication by the trichothecene mycotoxins in experimental animals are diarrhea, nausea, and decrease of spontaneous movement and body temperature. Histopathological changes of the gastrointestinal tract, spleen, thymus, etc. caused severe degeneration and necrosis within the tissue, “radiomimetic “(Sato and Ueno, 1977).

The signs and symptoms in Tables-1 and 2 were evaluated using Chi Square. The results are shown in (Chart 1). Clearly, all scores decrease to zero, which is attained only at death. The Wisconsin Case is at a Stage attained by the New York group occurring between 2000 and 2001. Because the New York residents still remain exposed to the mycotoxin, they are at imminent danger, high risk, as the data indicate they are approaching zero.

Discussion

Reviews of the four Cases, the urinary test confirmed the exposure to trichothecene mycotoxins as expressed by the signs and symptoms that identify this disease (Forgacs *et al.*, 1962; Joffe, 1971; Croft *et al.*, 1986). Mold trichothecene mycotoxin exposure can be established to a high degree of scientific certainty using the urine detection method of study. The signs, symptoms and severity of each were numerically recorded. This allowed for the establishment of the primary signs and symptoms, which included mental depression, narcolepsy, severe malaise and fatigue, severe headaches and others as shown on Table-1 for the four Cases. The scores generated in Table-1 and 2 and how they relate to death from this disease are found in Chart 1.

The brain, lung, cardiovascular, skin, intestine, immune, mucous membranes, joint and muscle, teeth and gums, eyes and ears are the target organs effected as shown in Table-1 and 2. (Forgacs *et al.,* 1962; Talmage 1983; Croft *et al.,* 1986; Wannemacher *et al.,* 1997). The patients were then classified into the three Stages of the disease. Their urine was assayed and confirmed to contain trichothecene mycotoxins, using TLC. The reproduction of the disease in animals fulfilled Koch's Postulates. This urine test confirms exposure to the causative poison and confirms the diagnosis of Trichothecene Mycotoxicosis.

Comparative pathology between man and animal clearly demonstrated human exposure via inhalation of the spores and mycotoxin, caused extensive loss of functional tissue due to inflammatory and caustic response to the mycotoxins. There was a severe loss of neurons of the brain in the cerebral hemisphere, as well as in the brain stem and Purkinje cells of the cerebellum (Forgacs *et al.,* 1962; Karppanen *et al.,* 1989). Lipid peroxidation (Hoehler *et al.,* 1998; Rizzo *et al.,* 1994) brain neurochemistry (Melocheme *et al.,* 1995; Porter *et al.,* 1995; Smith, 1992) biogenic amines (Prelusky, 1993) monamine oxidase activity and protein synthesis (Wang *et al.,* 1993; Wang *et al.,* 1998; Bergmann *et al.,* 1988; Bergmann, 1993; Bunner *et al.,* 1988; Cannon *et al.,* 1982; Busby *et al.,* 1981; Wyatt *et al.,* 1973) have been altered by trichothecene mycotoxin exposure within the brains of animals. The vessels of the brain in man and rats demonstrated severe degeneration, necrosis and infarction. Focal areas of necrosis within the cerebral hemisphere were also detected. There was a severe loss of neurons observed in the dorsal and ventral nervous tissue of the spinal cord, clearly establishing that the central nervous system is severely affected in man and animals. This observation has been made associated with ingestion of trichothecene mycotoxins in man and animal (Forgacs *et al.,* 1962; Joffe, 1971; Sato *et al.,* 1975). There was severe fibrinous inflammation within the human tissue, especially the lung, causing severe thickening of the interstitial tissue and decreased lung function. The Trichothecene mycotoxins essentially react with all cells of the human body based on signs, symptoms and pathology observed.

In Case No. 1, the Las Vegas family had extensive signs and symptoms although their clothing had been treated with 6% sodium hypochlorite to neutralize the mycotoxin. The urine and the Patch test clearly revealed that the mycotoxin was not altered and the people still remained exposed to high levels of mycotoxins, which are known to be cumulative in their poisoning effects (Joffe, 1971). This Case clearly demonstrated that 6% sodium hypochlorite does not inactivate the mycotoxin or destroy the allergy generating spores and should not be relied on to decontaminate a mold site. Porous items such as clothing, and carpeting, electronic equipment that cannot be cleaned or that are without a smooth surface must be discarded. Application of sodium hypochlorite (bleach) or any other antifungal agent in attempt to prevent mold growth, threatens mold survival and results in an increase of sporulation and mycotoxins produced at mold growth site (Talmage, 1983).

In Case No. 2, the Kentucky family mold exposure was identified by the urine test even though an obvious mold site was not observed. In Case 3, the female in DePere, Wisconsin clearly demonstrated that insulation should never be installed within ventilating ducts, because of the lack of moisture barriers allow moisture to accumulate and mold growth. in case 4 the building contains internal structural water and mold contamination not allowing for repair. This building must be condemned for health safety reasons. Destruction of such a building must be done a piece at a time and not by implosions due to release of large amounts of mold spores or contaminated dust release to the surrounding public population.

In all four Cases, the signs and symptoms expressed by the families establish a commonly recognized disease (Ueno, 1977). Identification of

the type of mold growing or spore counts could not establish exposure to the residents. The collection of urine from the four Case groups (30 residents), and the extraction of mycotoxin clearly define exposure to the trichothecene mycotoxins in each individual. The reproduction of the disease or poisoning within the animals (Croft *et al.*, 1986; Ueno, 1983; Joffe, 1971) fulfills Koch's Postulates (Umbreit, 1962), establishes the cause of the disease. The pathology observed within the New York resident confirms Koch's Postulates and inhalation exposure to the trichothecene mycotoxin. Each poison expresses a fingerprint, as signs and symptoms or pathology to confirm the causative agent (Cheville, 1976). Any person found with mycotoxin released within their urine due to exposure to a mold-contaminated building, is advised to avoid further exposure because of irreversible brain, spinal cord, lung, heart, liver, kidney, pancreas, intestine and eye damage.

An undeterminable "dose" of any chemical never has relevance to the diagnosis or safety (Edwards, 1979; Peters *et al.*, 1984; Croft *et al.*, 1986) because of multiple chemical exposures and multiple interactive responses, additive or exponential effects (Casarett and Doull, 1991, 1996).

The mycotoxin can be released as a vapor in which spore identification or spore counts are not able to establish exposure, but a urine sample will express exposure to vapors of the mycotoxin detected within the urine. The excretion of trichothecene has been examined in several species of animals. These data indicate that most of the mycotoxin administered in levels substantially less than the LD_{50} is eliminated relatively quickly through the feces and urine (Talmage, 1983; Wannemacher, 1997). In this study the levels of mycotoxin excreted from the urine reflect exposure levels; the more mycotoxin extracted, the higher the exposure. The test for mycotoxins in the urine becomes a useful tool to confirm exposure to trichothecene mycotoxins, whether at low levels over a chronic period, or high levels in acute situations in patients.

The use of sight or mold spore counts used to detect a mold-contaminated building has been shown to be very inaccurate especially when mold is growing within internal structure or under carpeting (Burge *et al.*, 2000). The urine test could be used in public buildings such as schools, where children are 100-1000 times more susceptible than adults to the poisoning effects, to confirm or deny exposure to the bio-terrorist, trichothecene mycotoxins due to the sensitivity and accuracy (Talmage, 1983; Wannemacher, 1997). U.S. EPA reports that 50% of the U.S schools are suffering from indoor air quality problems, or 25 million students are affected as of 2002. Children exposed to any level of trichothecene mycotoxins are in imminent danger and should not enter any contaminated building at any cost. Clothing worn by children will contaminate the home of a child while attending a mold-contaminated building such as a school and home contents must be discarded as clearly demonstrated in Case No. 1 of this report.

The Patch Test is another useful method to use when patients expressing signs and symptoms related to mold conditions are initially contacted. The mycotoxin inhibits alcohol dehydrogenase (Ueno, 1977), a very common enzyme (Casarett and Doull's, 1996) as with other SH-residues of SH-enzymes and can be used to detect level of exposure in most Cases. The limitations are genetic production (Higuchi, *et al.*, 1987) as observed in the Mongoloid population and other chemical epoxides exposure (Ueno, 1977) or disulfiram (Casarett and Doull's, 1996).

The use of skin or Rast testing to detect exposure to molds has been utilized, but these tests do not date the exposure and may have false negatives. These tests can be used in treating patients for their allergies and in some Cases, life-threatening conditions after they are removed from contaminated environments.

The urine test appears to be the method to establish exposure and confirm or deny the diagnosis of mycotoxicosis caused by trichothecene mycotoxins in man, especially in Cases where exposure or sources are at question.

People Exposure to Trichothecene mycotoxins via the inhalation route is very poisonous to the human body.

Acknowledgements

Work was supported by Environmental Diagnostic Group Inc. Madison, Wisconsin. Special thanks goes to JoAnne Croft for review and technical support of this study and to Nancy A. Didriksen, Ph.D. for a review of this study. To Drs. Bruno Schiefer and Judith Smits, for their mold knowledge, experiences shared and mentoring ability. To Dr. Bruce Jarvis for his kind input into analysis of trichothecene mycotoxins from time to time. All efforts by the above are greatly appreciated.

References

Bergmann, F., D. Soffer and B. Yagen : Cerebral toxicity of the trichothecene toxin T-2, of the products of its hydrolysis and of some related toxins, *Toxicon.*, **26**, 923-930 (1988).

Bergmann, F. : Toxicological studies with trichothecenes, applied to the rat brain *in vivo* and *in vitro. J. Toxicol*, *Toxin Reviews*, **12**, 91-102 (1993).

Best, C. H., and N. B. Taylor : The physiological basis of medical practice, A Text in applied physiology, 7th Edition, The Williams & Wilkins Company, pp, 1286-1289 (1961).

Busby, Jr. W. F. and G. N. Wogan : Trichothecenes. *In* : Mycotoxins and N-Nitroso compounds : Environmental risks, Vol II, Chapter **3**, 30-45 (1981).

Bunner, D. L. and E. R. Morris : Alteration of multiple cell membrane functions in L-6 myoblasts by T-2 toxin : An important mechanism of action. *Toxicol. Appl. Pharmacol.,* **92**, 113-121 (1988).

Burge, H. A., D. L. Pierson, T. O. Groves, K. F. Stawn and S. K. Mishra : Dyanmics of airborne fungal populations in a large office building. *Current Microbiol.*, **40**, 10-16 (2000).

Cannon, M., W. I. Cranston, R. R. Hellon and Y. Townsend : Inhibition by trichothecene antibiotics of brain protein synthesis and fever in rabbits. *J. Physiol*., **322**, 447-455 (1982).

Casarett and Doull's : Toxicology, The Basic science of poisons, 4th Edition, Pergamon Press, pp, 12-30 (1991).

Casarett and Doull's : Toxicology, The Basic science of poisons, 5th Edition, Curtis D. Klaassen, McGraw-Hill, Health Professions Division, pp, 122, 751 (1996).

Costantini A.V., H. Wieland and L. I. Qvick : Fungalbionic Series, The Fungal / Mycotoxin etiology of human disease, Etiology and prevention of prostate cancer hope at last. Johann Friedrick Oberlin Verlag, Freiburg, Germany (1998).

Costantini A.V., H. Wieland and L. I. Qvick : Fungalbionic Series, The Fungal / Mycotoxin etiology of human disease, prevention of breast cancer hope at last, Johann Friedrick Oberlin Verlag, Freiburg, Germany (1999).

Croft, W. A., B. B. Jarvis and C. S. Yatawara : Airborne Outbreak of trichothecene toxicosis, *Atmospheric Environ.*, **20**(**3**)., 549-552 (1986).

Cheville, N. F. : Cell pathology, The Iowa State University Press / Ames, pp 3 (1976).

Edwards, W. C. : The diagnosis of petroleum hydrocarbon poisoning in cattle. *Vet. Med. Small Anim. Clinic.*, **74,** 1516-1518 (1979).

Forgacs, J., and W. T. Carll : Mycotoxicoses. *In* : Advances in Veterinary Science. Academic Press, New York and London, pp 273-372 (1962).

Higuchi, S., T. Muramatsu, M. Sato, M. Sasao, K. Maruyama and H. Kono : Ethanol Patch test for low Km aldehyde dehydrogenase deficiency. *The Lancet*, 1**4**, 629. (1987).

Hoehler, D., R. R. Marquardt, A. R. McIntosh and S. Madhyastha : Free radical-mediated lipid peroxidation induced by T-2 toxin in yeast (Kluyveromyces marxianus). *J. Nutr. Biochem.*, **9**, 370-379 (1998).

Joffe, A. Z. : Alimentary toxic Aleukia. *In* : Microbial toxins, Vol. VII (*Ed* : S. Kadis, A. Ciegler and S.J. Ajl). pp, 139-189. Academic Press Inc., New York. (1971).

Johanning, E. : Bioaerosols, Fungi and Mycotoxins : Effects, assessment, prevention and control. *Eds* : Eastern New York Occupational and Environmental Health Center, Albany, New York, 12-21 (1999).

Karppanen, E., A. Rizzo, L. Saari, S. Berg and H. Bostrom : Investigation on trichothecene-stimulated lipid peroxidation and toxic effects of trichothecenes in animals. *Acta Vet. Scand*., **30**, 391-399 (1989).

Meloche, J. L. and T. K. Smith : Altered tissue amino acid metabolism in acute T-2 toxicosis, P.S.E.B.M., **210**, 260-265 (1995).

Pasanen, A. L., S. Lappalainen and P. Pasanen : Volatile organic metabolites associated with some toxic fungi and their mycotoxins. *Analyst.*, **121**., 1949-1953 (1996).

Pathre, S. V. and C. J. Mirocha : Assay methods for trichothecenes and review of their natural occurrence, mycotoxins in human and animal health (*Eds* : J.V. Rodricks, C.W. Hesseltine and M. A. Mehlman). Pathotox Publishers, Park Forest South, Ill. Page 229-253 (1977).

Peters, Henry, A., W. A. Croft, E. A. Woolson, B. A. Darcey and M. A. Olson : Seasonal arsenic exposure from burning chromium-copper-arsenate-treated wood. *JAMA*, **251**, 2393-2396 (1984).

Prelusky, D. B. : The effect of low-level deoxynivalenol on neurotransmitter levels measured in pig cerebral spinal fluid. *J. Environ. Sci. Hlth.*, **6**, 731-761 (1993).

Porter, J. K., C. W. Bacan, E. M. Wray and W M. Hagler Jr. : Fusaric acid in Fusarium moniliforme cultures, corn, and feeds toxic to livestock and the neurochemical

effects in the brain and pineal gland of rats. *Natural Toxins*, **3**, 91-100 (1995).

Rizzo, A. F., F. Atroshi, M. Ahotupa, S. Sankari and E. Elovaara : Protective effect of antioxidants against free radical-mediated lipid peroxidation induced by DON or T-2 toxin. *J. Vet Med. A.*, **41**, 81-90 (1994).

Robins, S. L. : Pathology, 3rd Ed., W. B. Saunders Company, Philadelphia, London pp 55 (1969).

Robison, T. S., C J. Mirocha, H. J. Kurtz, J. C. Behrens, G. A. Weaver and M. S. Chi : Distribution of tritium-labeled T-2 toxin into swine. *J. Agric. Food Chem.*, **27**, 1411-1413 (1979).

Steel, G.D. and J.H. Torrie : Principles and procedures of statistics, with special reference to the biological sciences. McGraw-Hill Book Company, Inc., New York (1960).

Sato, N., Y. Ueno and M. Enomoto : Toxicological approaches to the toxic metabolites of Fusaria, VIII. Acute and subacute toxicities of T-2 toxin in cats, Japan. *J. Pharmacol.*, **25**, 263-270 (1975).

Sato, N., Y. Ueno : Comparative toxicities of trichothecenes. *In* : Mycotoxins in human and animal health (*Eds*. : J.V. Rodricks, C. W. Hesseltine and M. A. Mehlman). Pathotox Publishers, Inc. pp 295-307 (1977).

Smith, T. K. : Recent advances in the understanding of Fusarium trichothecene mycotoxicosis. *J. Animal Sci.*, **70**, 3989-3993 (1992).

Talmage, D. W. : Protection against trichothecene mycotoxins. Committee on protection against mycotoxins, Board on Toxicology and Environmental Health Hazards, Commission on Life Sciences, and National Research Council, National Academy Press, Washington, D. C. pp, 128-129 (1983).

Ueno, Y. : Trichothecenes : overview address. *In* : Mycotoxins in human and animal health (*Eds.* : J. Rodricks, C. W. Hesseltine and M. A. Hehlman). Pathotox, Inc., Park Forest South, IL., pp, 189-207 (1977).

Ueno, Y. : Trichothecene mycotoxins-mycology, chemistry, and toxicology. *Adv. Nutr. Sci.*, **3**, 301-353 (1980).

Ueno, Y. : Trichothecenes-chemical, biological and toxicological aspects. *Devel. Food Sci.*, **4**, Elsevier, New York (1983).

Umbreit, W. W. : Modern microbiology, The Basic tools of microbiology, pp 30, (1962).

Wang, J., D. W. Fitzpatrick and J. R. Wilson : Effect of dietary T-2 toxin on biogenic monoamines in discrete areas of the rat brain. *Fd. Chem. Toxic.*, **31**, 191-197 (1993).

Wang, J., D. W. Fitzpatrick and J. R. Wilson : Effects of the trichothecene mycotoxin T-2 toxin on neurotransmitters and metabolites in discrete areas of the rat brain. *Food Chemi. Toxicol.*, **36**, 947-953 (1998).

Wannemacher, R. W. Jr. and S. L. Wiener : Chapter 34 Trichothecene mycotoxins, *In* : Text Book of Military Medicine aspects of chemical and biological warfare. pp 655-677 (1997).

Wyatt, R. D., W. M. Colwell, P. B. Hamilton and H. R. Burmeister : Neural disturbances in chickens caused by dietary T-2 toxin. *Appl. Microbiol.*, **26**, 757-761 (1973).

Correspondence to :
Dr. William A. Croft, DVM, Ph.D.,
521 Hilltop Drive, Madison, Wisconsin 53711 (USA).
Fax : 715-757-9302
E-mail : doccroft@hotmail.com